Interactions of Chemical Warfare Agents with Acetylcholinesterase

DoD Challenge Project
FY02

Project Leaders

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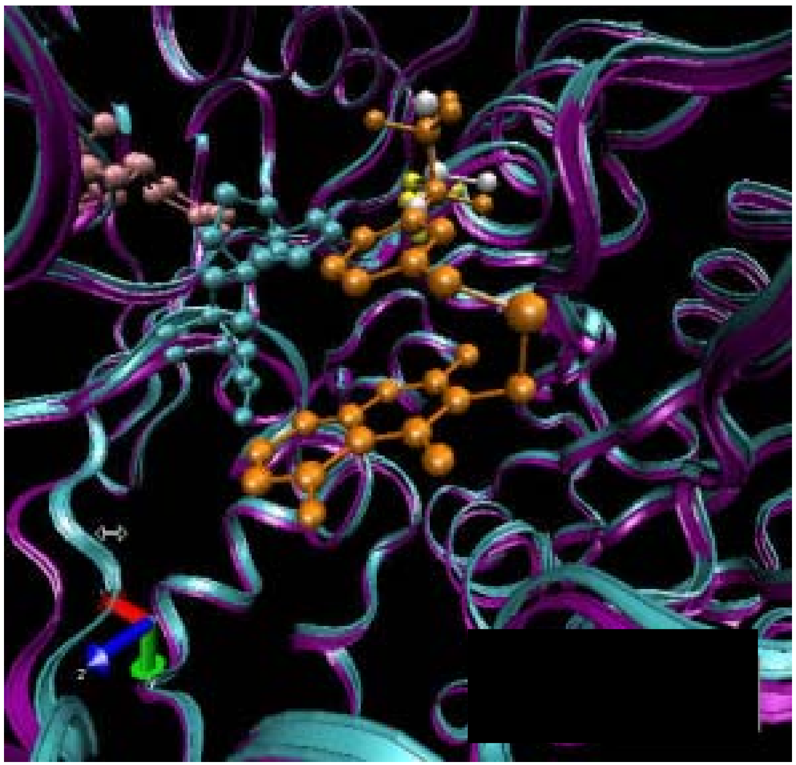
Technical Goals

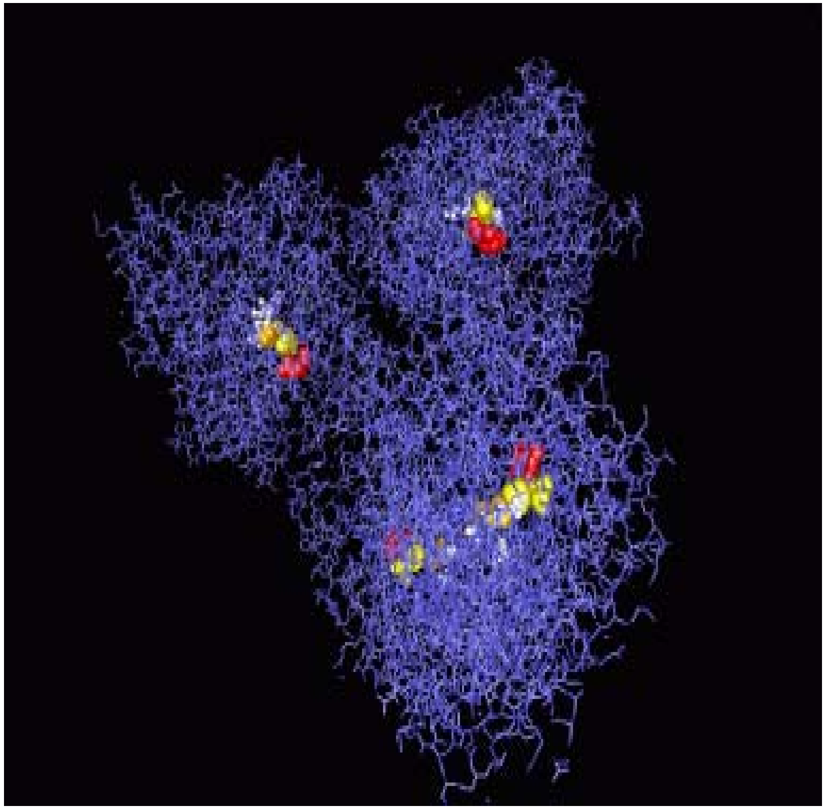


Objective: Understanding of

- **reversible and irreversible binding by organophosphorus and other compounds in active site of acetylcholinesterase**
- **role of solvent**
- **mechanism of oxime therapy**
- **‘Aging’ mechanism**

by theoretical modeling to facilitate development of new compounds for therapeutics and prophylaxis, as well as understanding of new threats

Technical Goals



The Problem:

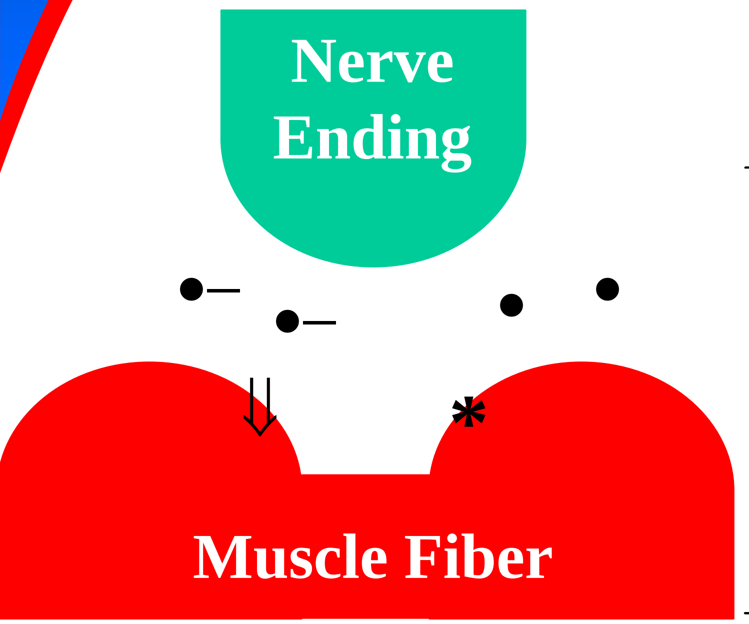
•Experiments are expensive, dangerous, and often technically limited

•Previous theoretical work is limited in size/level of accuracy

Therefore: Novel agent/antidote formulations are made by extrapolation from analogs

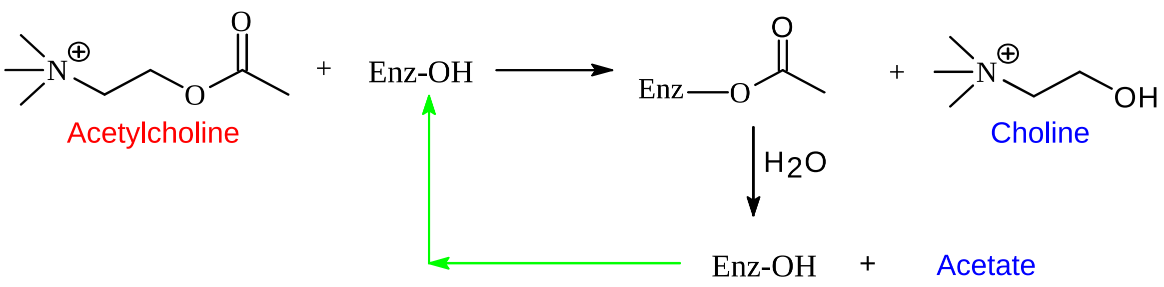
The Proposed Solution: QM/MM calculations as a means of studying nerve agent defensive mechanisms

Acetylcholinesterase (AChE)

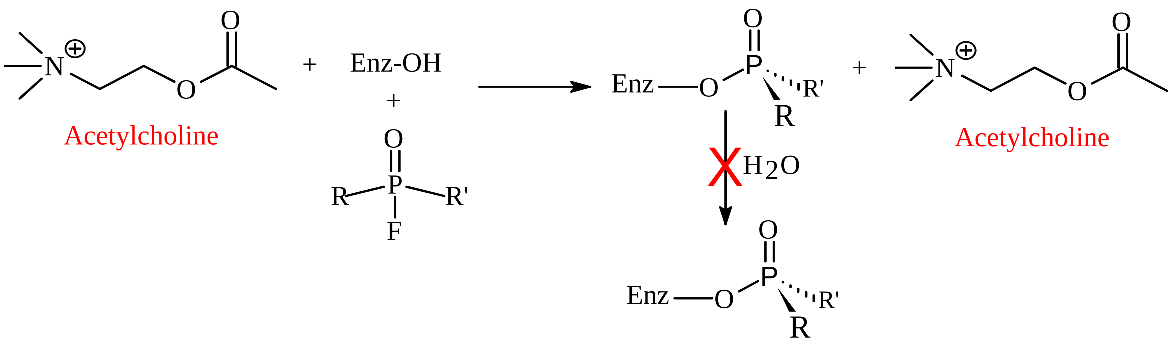


●— Acetylcholine
● Choline
⇓ Cholinergic Receptor
* AChE

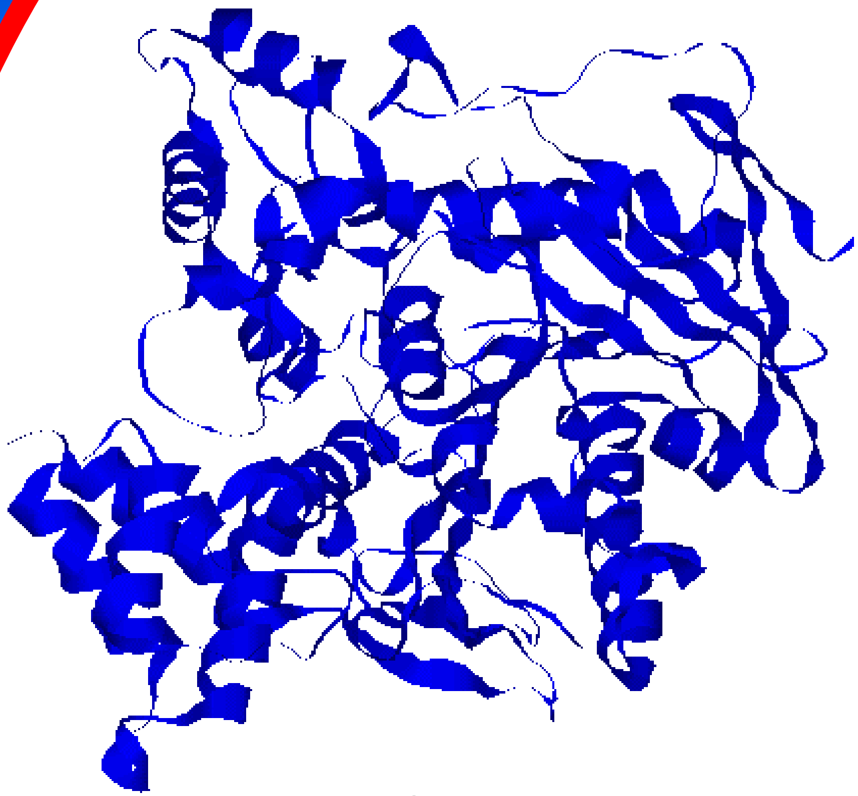
Normal AChE Function



Nerve Agent Deactivation

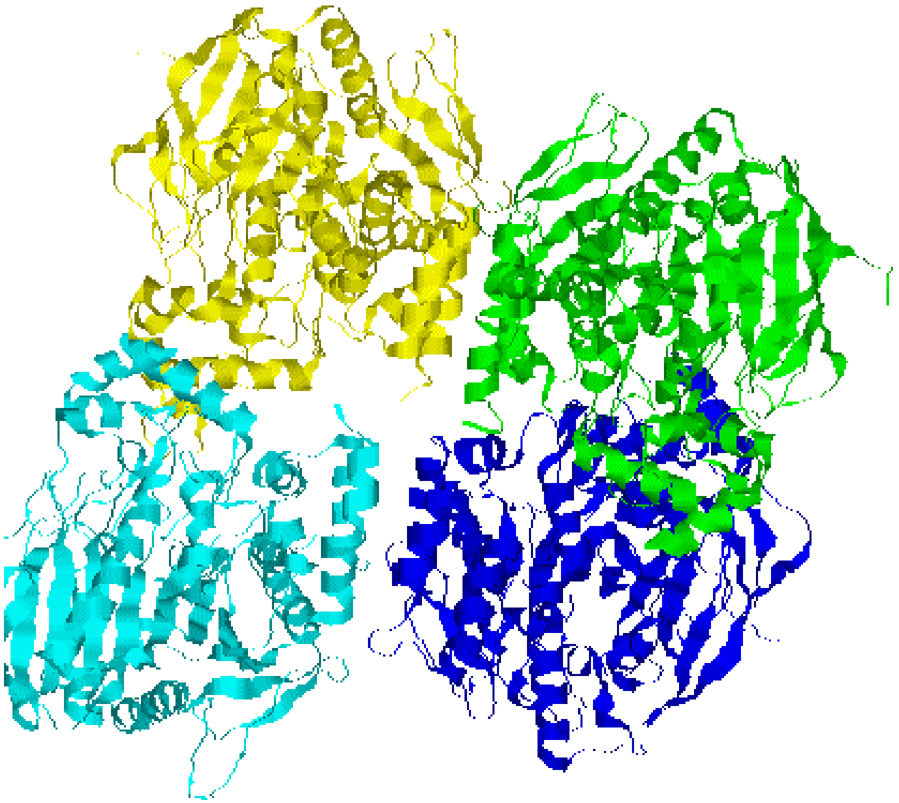


Acetylcholinesterases



Torpedo Californica – 1AMN

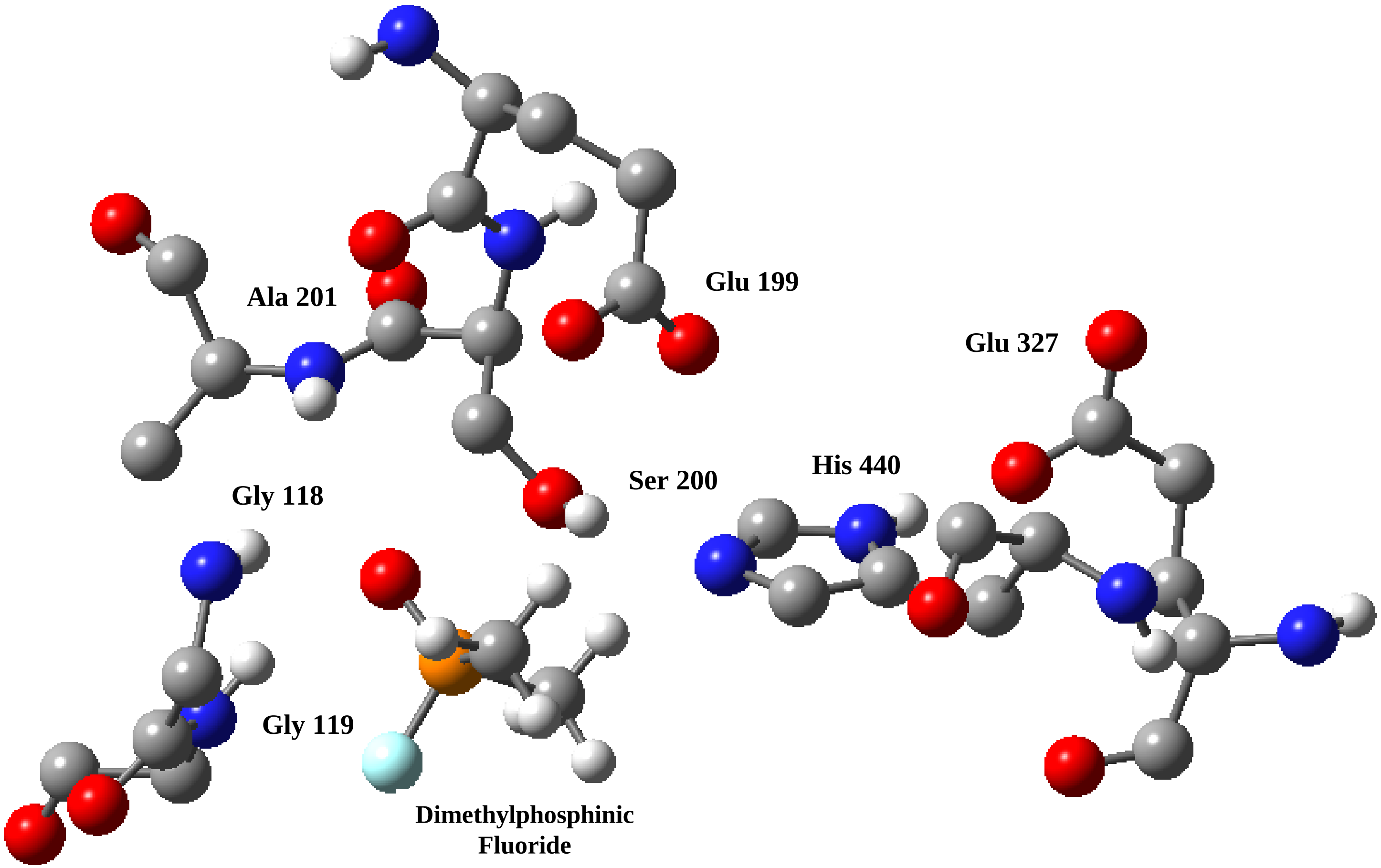
Harel, M., Quinn, D. M., Nair, H. K., Silman, I., Sussman, J. L.: The X-ray structure of a transition state analog complex reveals the molecular origins of the catalytic power and substrate specificity of acetylcholinesterase. J Am Chem Soc 118 pp. 2340 (1996)



Mus musculus – 1MAA

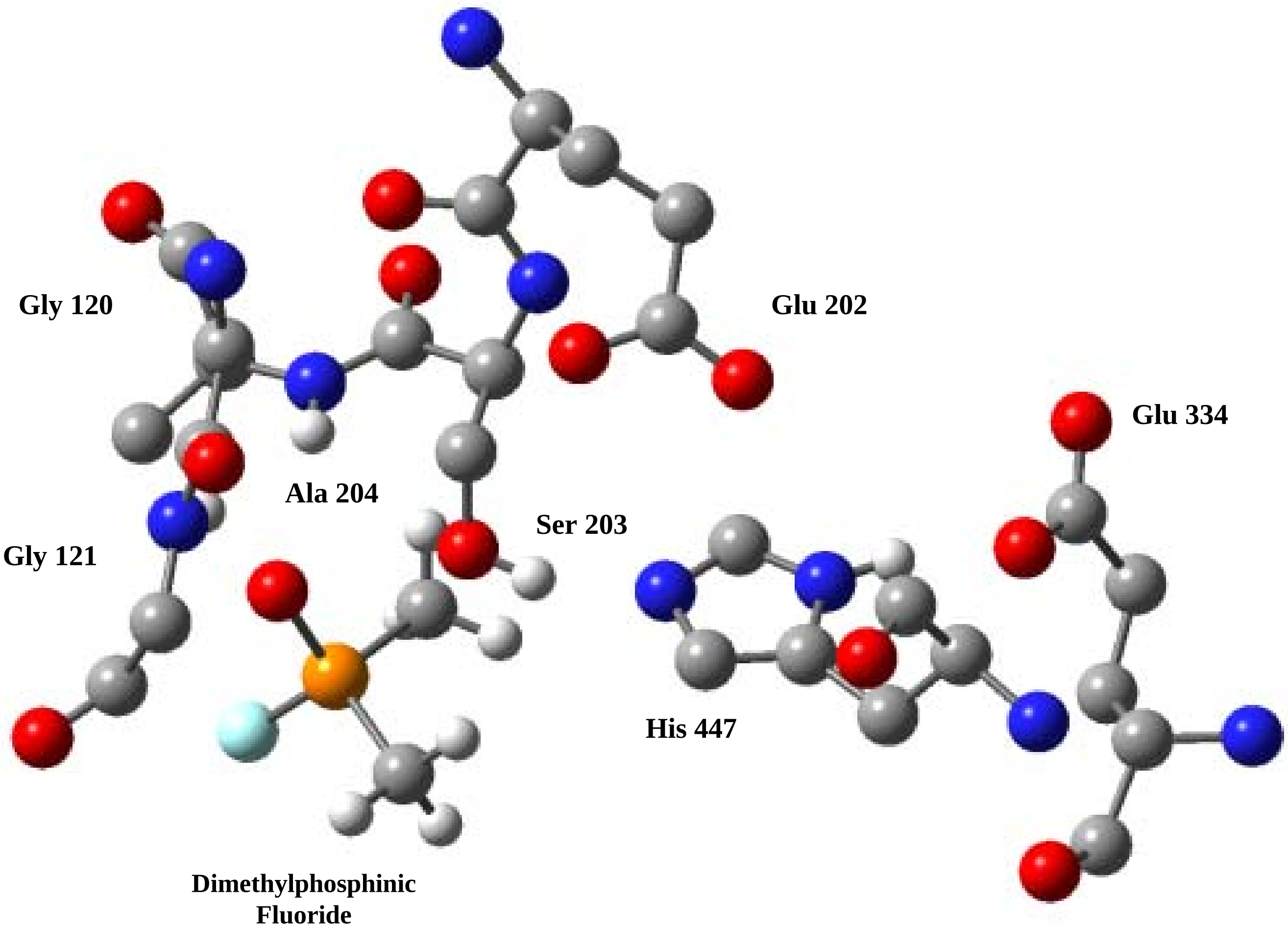
Bourne, Y., Taylor, P., Bougis, P. E., Marchot, P.: Crystal structure of mouse acetylcholinesterase. A peripheral site- occluding loop in a tetrameric assembly. J Biol Chem 274 pp. 2963 (1999)

Acetylcholinesterases



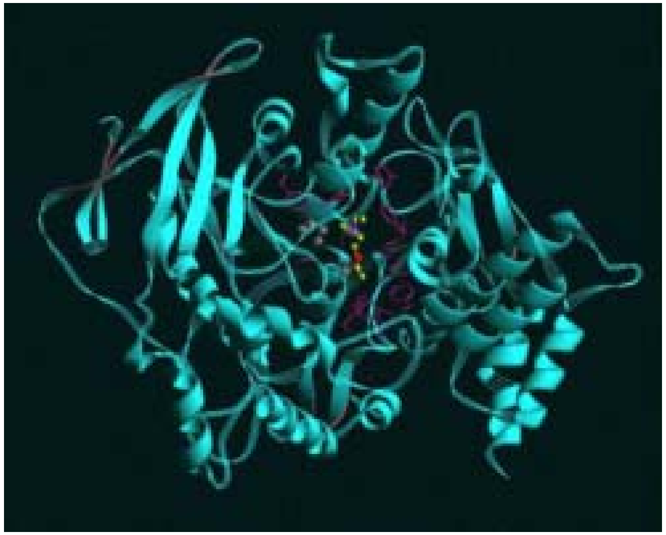
Torpedo Californica

Acetylcholinesterases

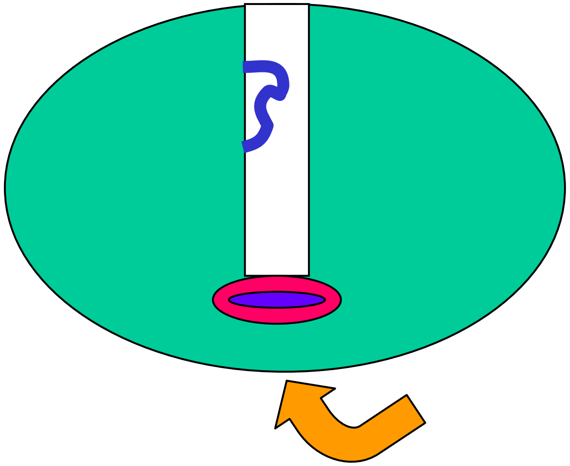


Mus Musculus

Acetylcholinesterase and Issues



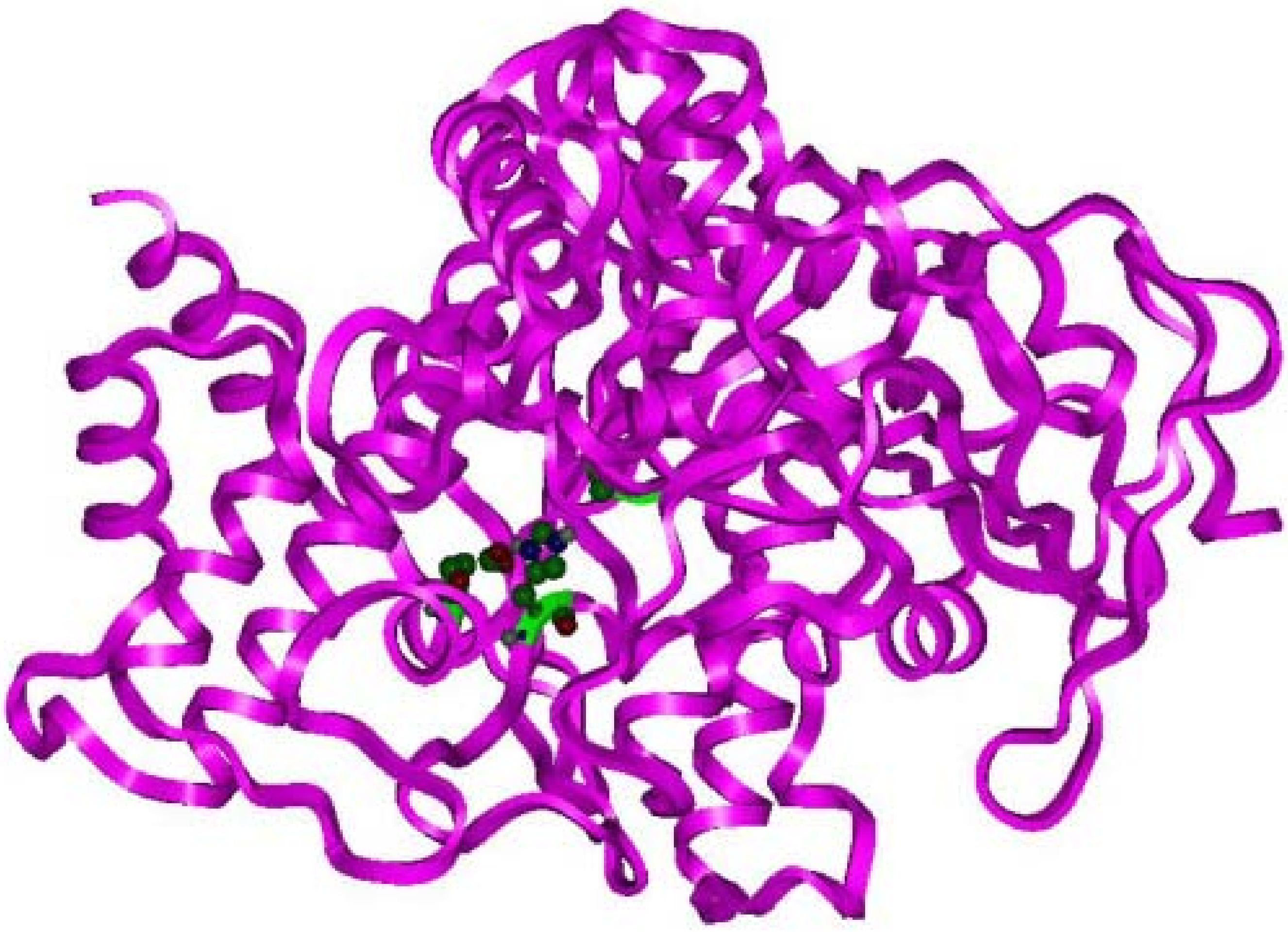
http://www.weizman.ac.il/Structure1_biology/Pages/Sussman



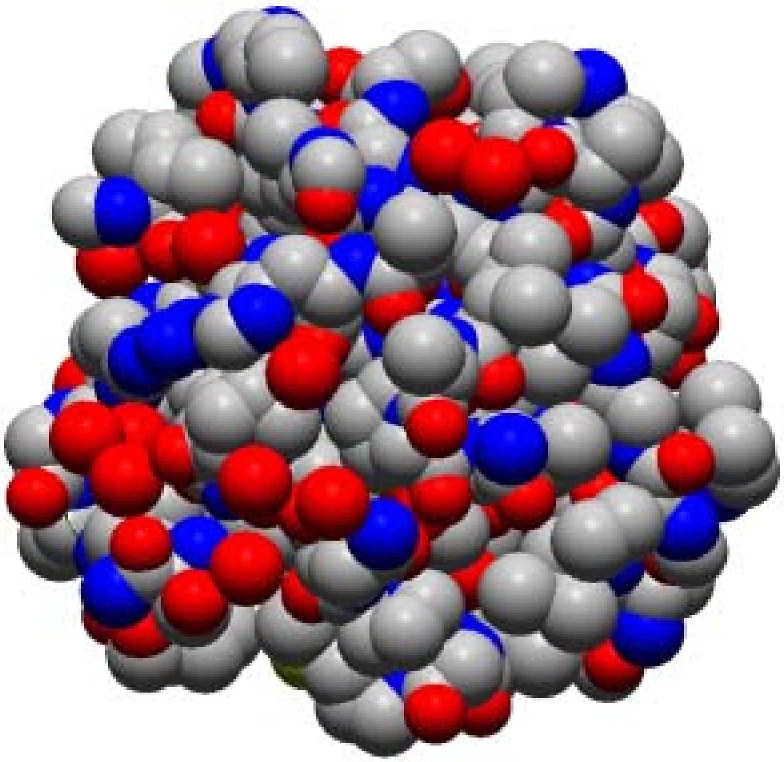
Issues surrounding the enzyme:

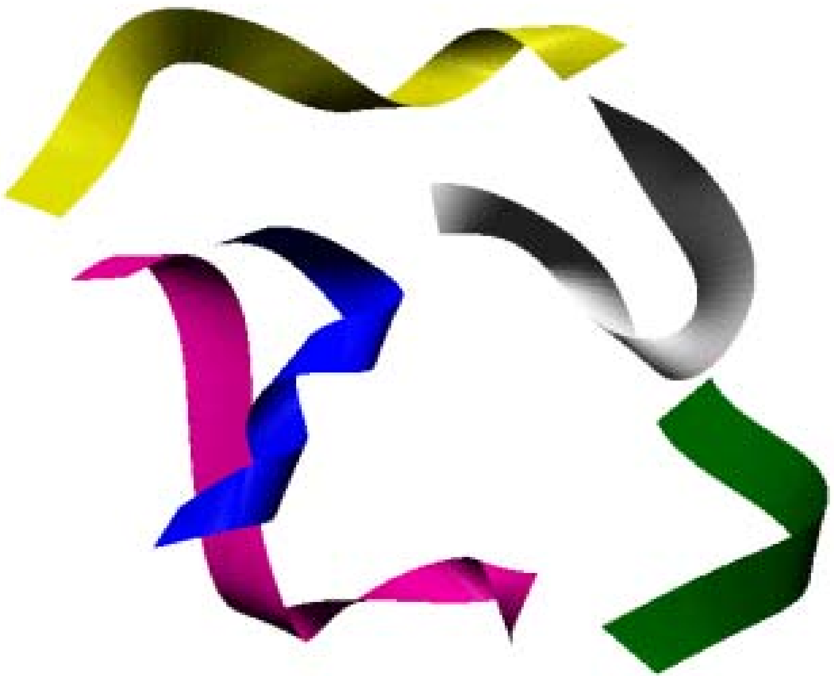
- Size
- Active site at bottom of gorge
- Role of solvent?
- "Back door" mechanism?
- Role of mobile loop?
- Role of surrounding residues?

'Ribbon Methodology'



‘Ribbon Methodology’

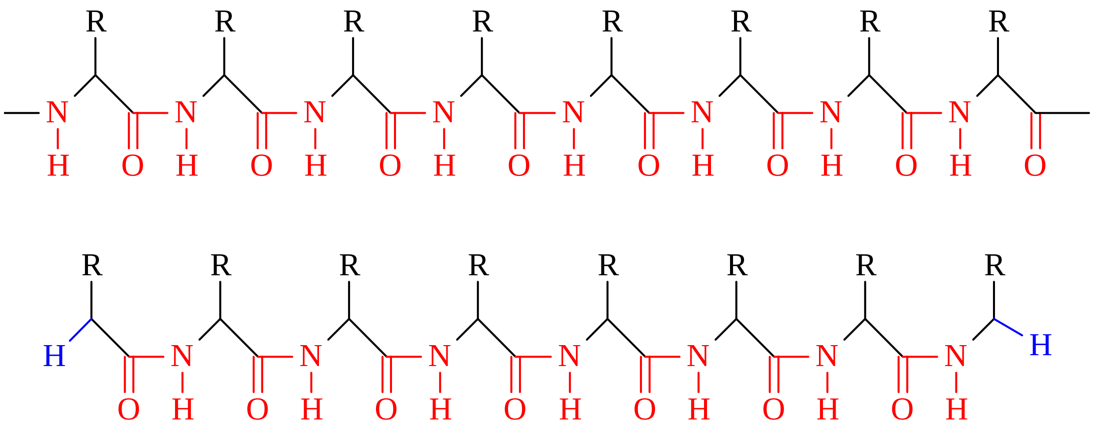




- **Eliminates the need to use expensive solvation methods since a predefined layer of enzyme atoms surrounds the active site chemistry being investigated.**

Setting up the Sphere

Termination of the ribbon ends consists of:



- **Keep the amide links with a C-R on either side**
- **Lock the C atom in space**

QM/MM and QM/QM Methodologies

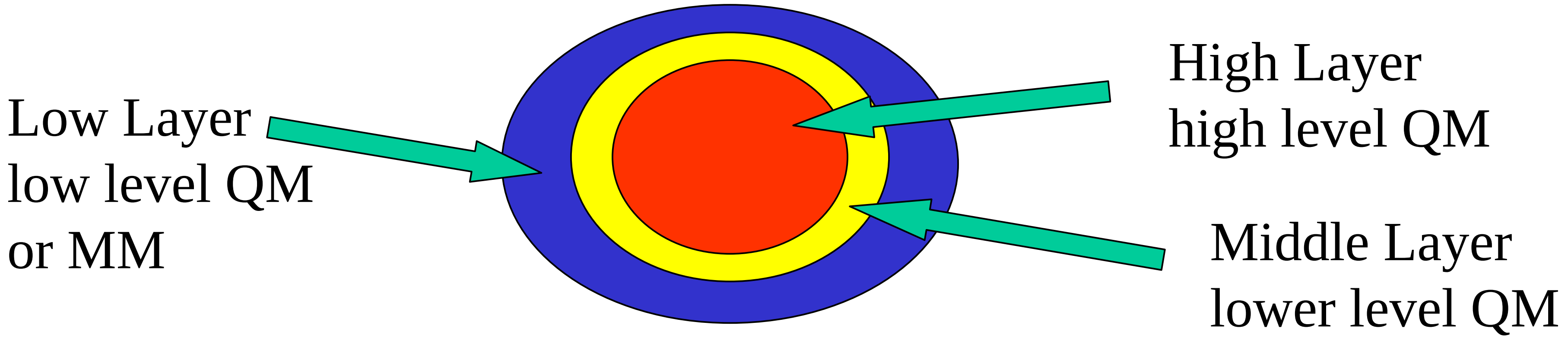
Tremendous advances have been made in QM/MM methodology in recent years. Two techniques have been chosen for our initial studies:

1. **We begin by using Morokuma's ONIOM method implemented in the G98 package.**
2. **For the sake of comparison of methodology, we also use the SIMOMM method of Shoemaker, Burggraf, and Gordon, which unites the GAMESS and TINKER codes.**

- **Allows treatment of larger area of interest than plain QM**
- **Allows more 'presence' of enzyme than traditional gas phase QM**
- **Higher level treatment than traditional MM, including reaction energetics, TS search, etc.**

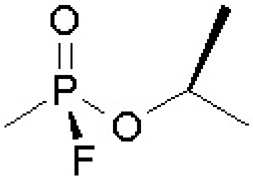
Comparison of Codes

ONIOM	SIMOMM
•extrapolative	•extrapolative
•UFF,DREIDING,AMBER	•AMBER, charmm, tinker,…
•2 or 3 layer	•2 layer
•E = E3 - E1 + E2	•E = EMM + EQM
•More fixed variables	•Fewer fixed variables
•Generic design	•Designed for surfaces

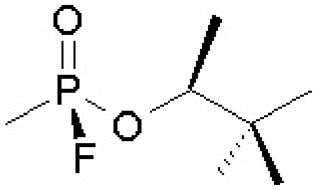


Nerve Agents

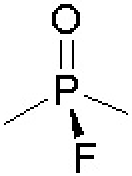
Sarin



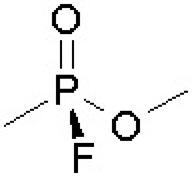
Soman



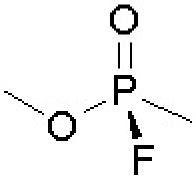
Test Cases:



phosphinate



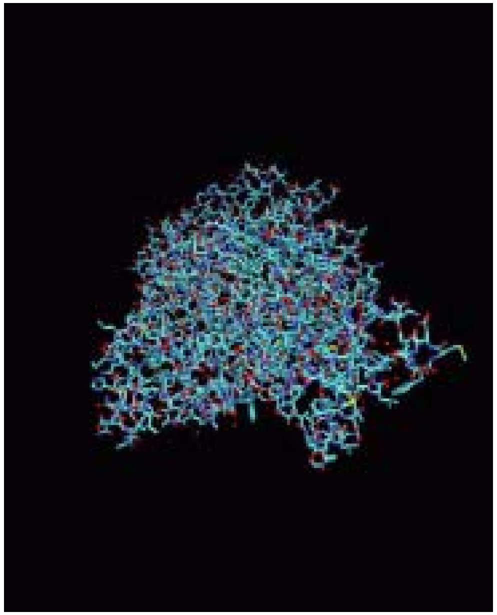
s-phosphonate

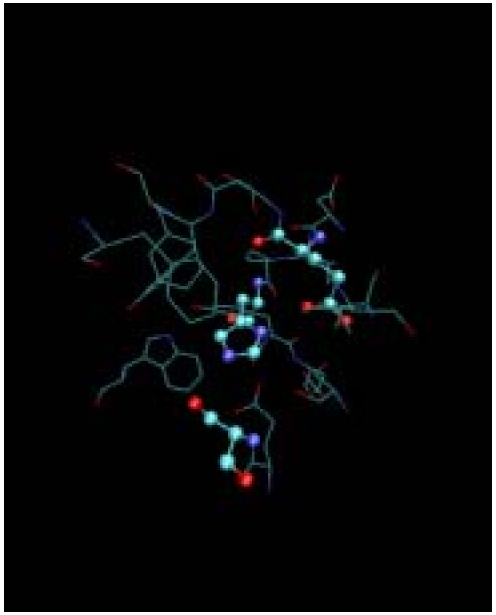


r-phosphonate

The Model

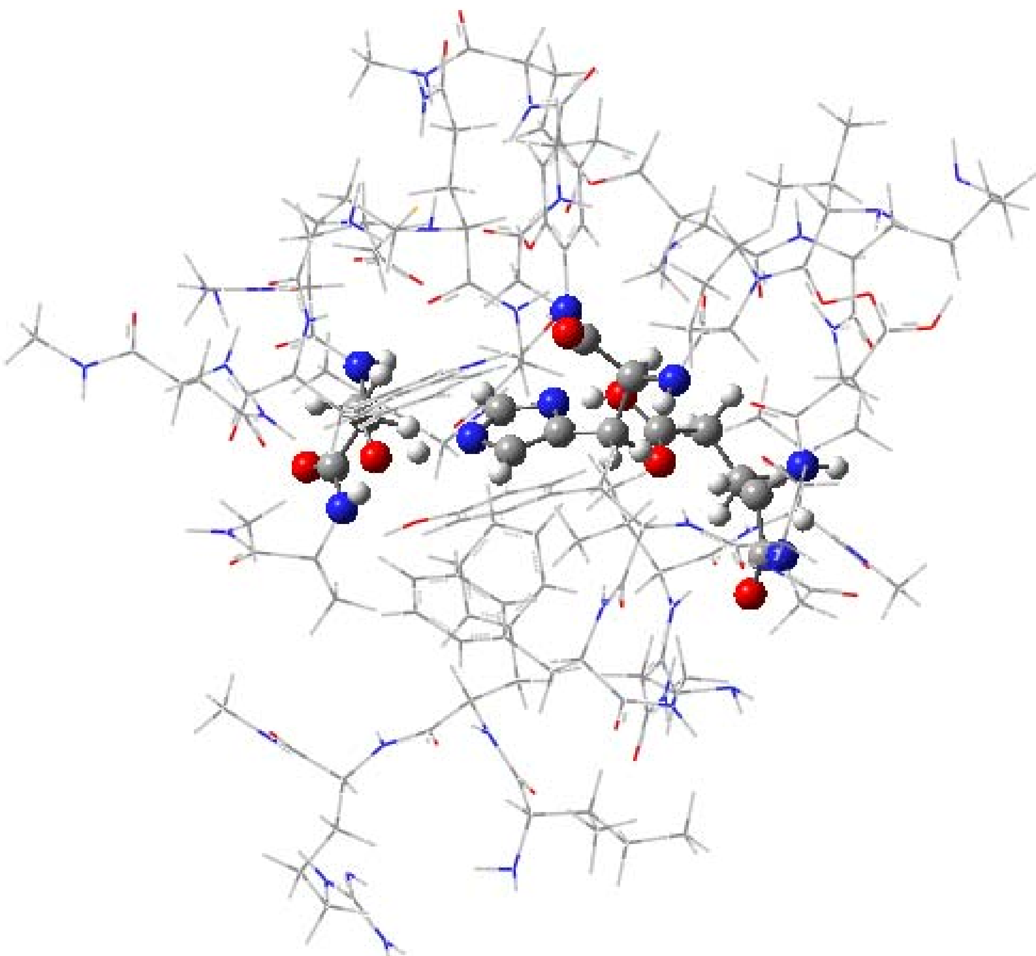
Model system is still truncated compared to true enzyme, but contains more features of real system than previous models.





Started by taking various size cuts (7 Ang and 10 Ang) around active site residues. Active site residues treated by QM, remainder by MM (initially)

QM/MM Bare Enzyme Model

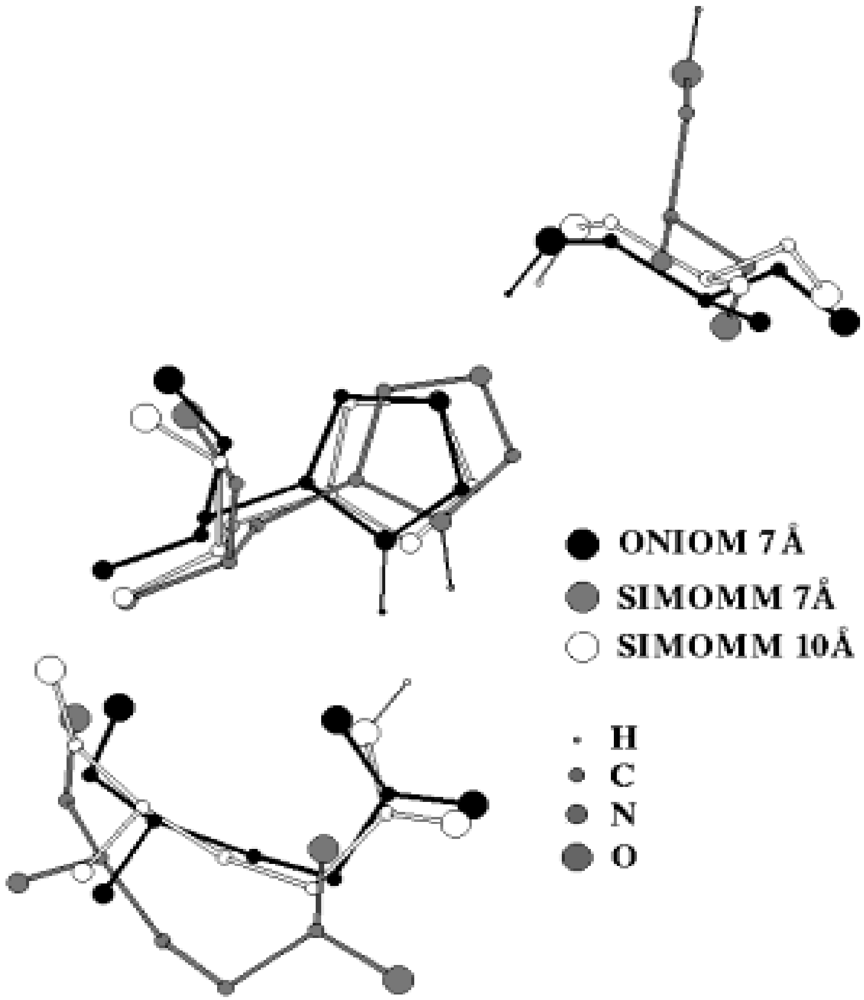


G98 result
Bare enzyme
Active site residues QM, remaining residues MM

Note alignment of active site for dual proton transfer

7Å Mouse AChE
254 heavy atoms, 250 H's
QM = B3LYP/STO-3G
MM = UFF

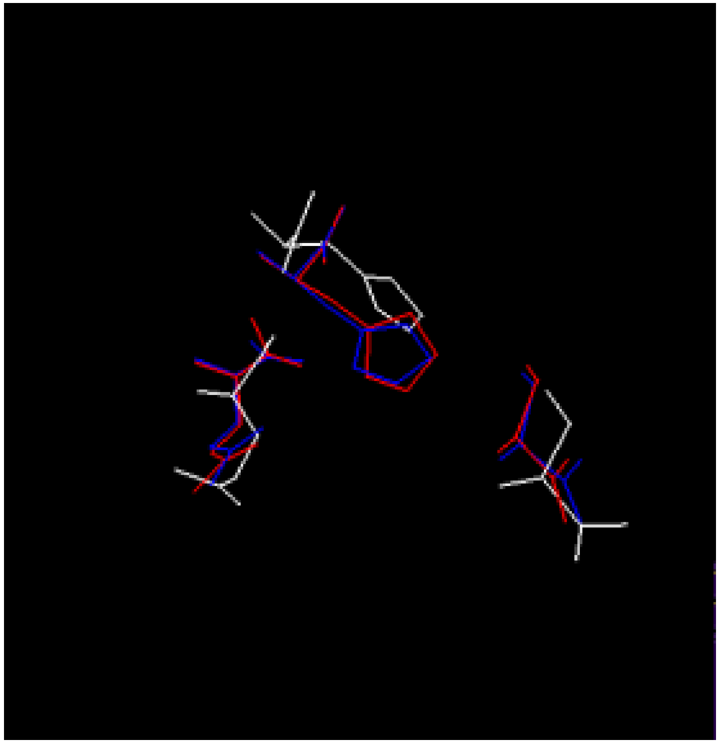
QM/MM Methodology?



- Significant difference in 7 A ONIOM vs SIMOMM
- SSBH? YES!
- Convergence difficulties with ONIOM
- No constraints necessary in ONIOM, model does not decohere
- Significant Difference in 7A vs 10A SIMOMM

MM Effect of FF?

Effect of Forcefield on Active Site Geometry?



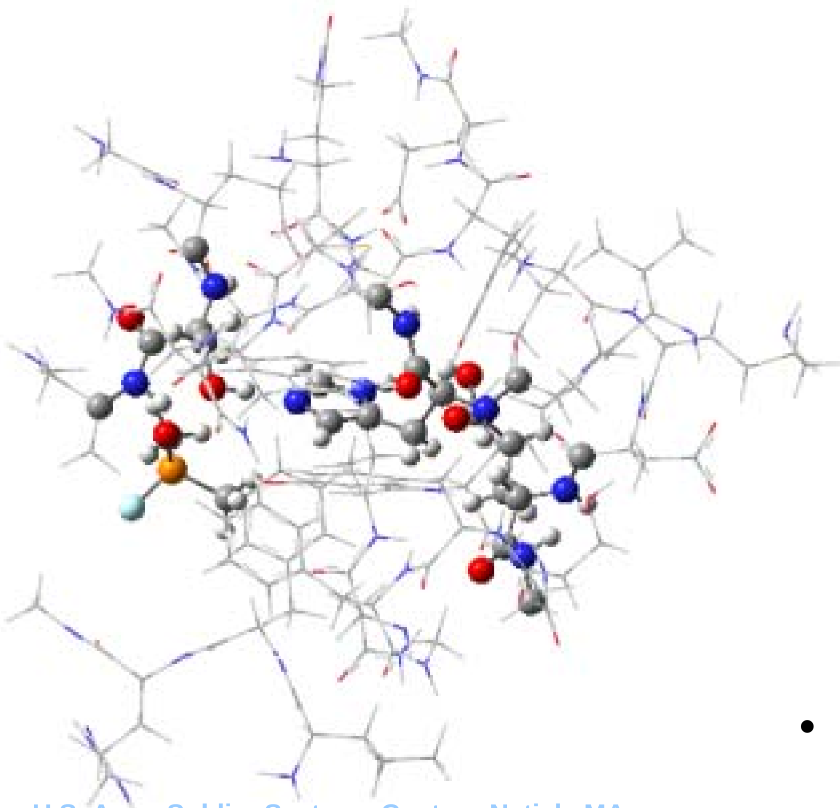
7 Ang Model, structure of active site (quantum region) only is shown to display effect of MM forces reflecting back into quantum regime

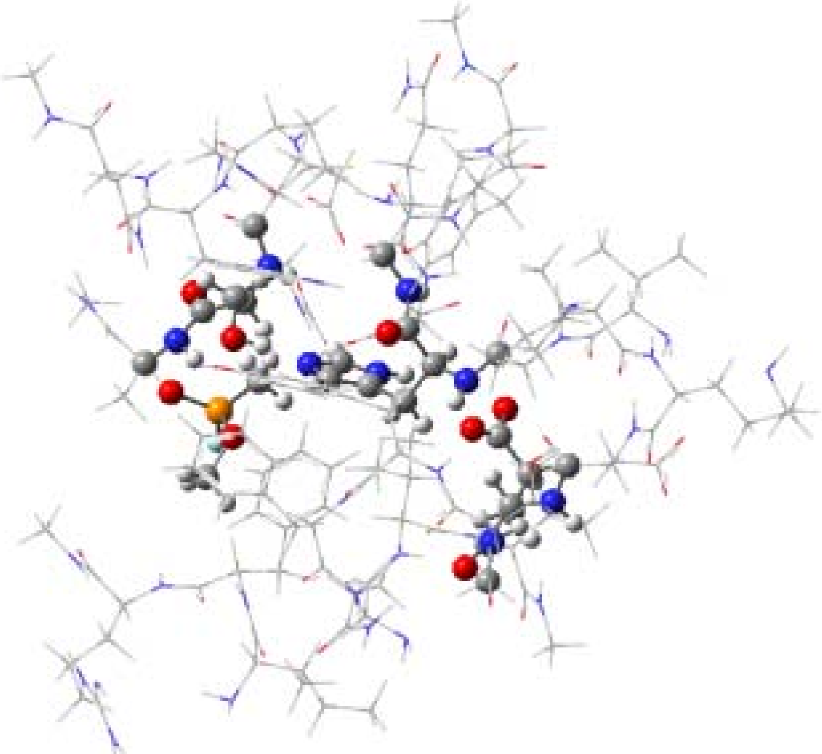
•AMBER FF (in white)
•DREIDING FF (in red)
•UFF (in blue)

Structural differences are minimal for DREID/UFF using ONIOMM, AMBER doesn't align as well
Problem w/handling of electrostatics at QM/MM interface?

Nerve Agent Activity

Phosphinate





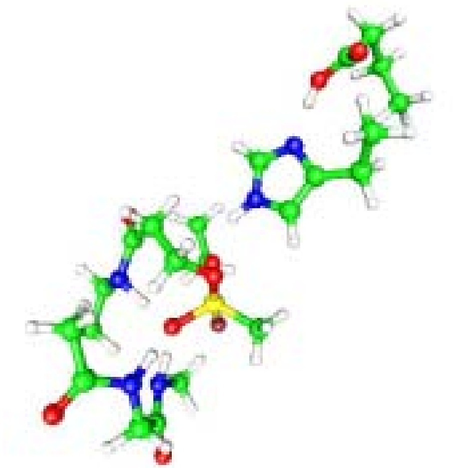
Phosphonate

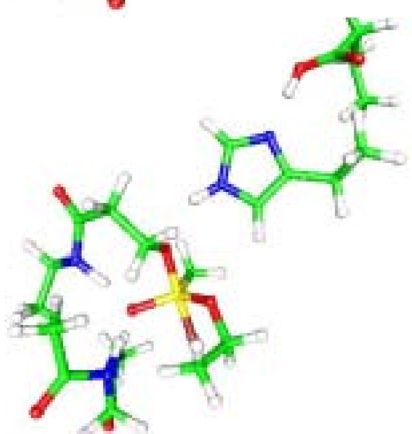
- Energetics underway with minimal and 7 angstrom model

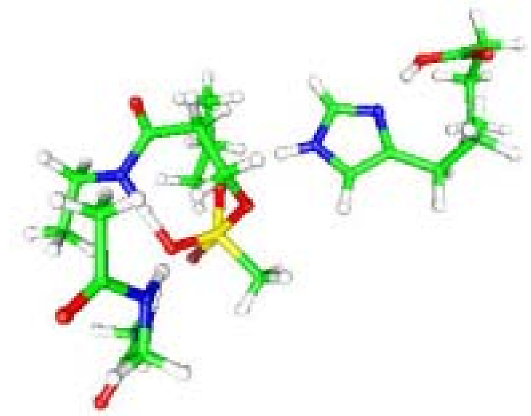
Nerve Agent Chirality: R vs S?

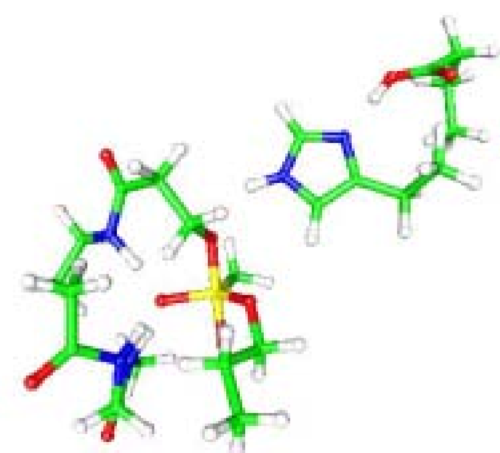
oniom=(B3LYP/6-31G(d,p):

B3LYP/sto-3G)









R vs S phosphonate – optimized products for ethyl, propyl, isoproyl alkoxy moiety preparatory to study aging

•No F- leaving?

•Similar Energy Differences?

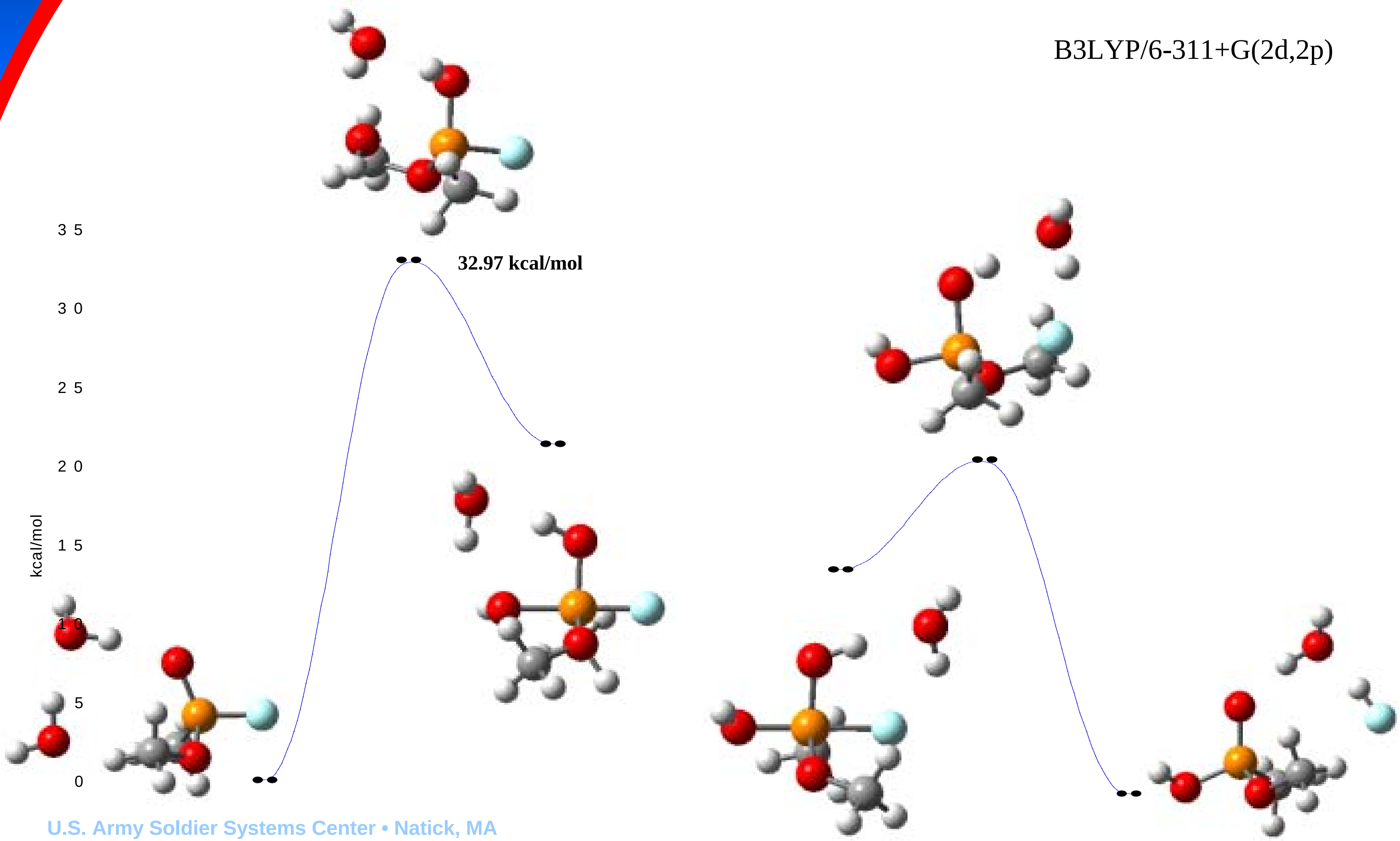
•Bumpy PES, multiple minima w/chain orienation

R and S ethyl

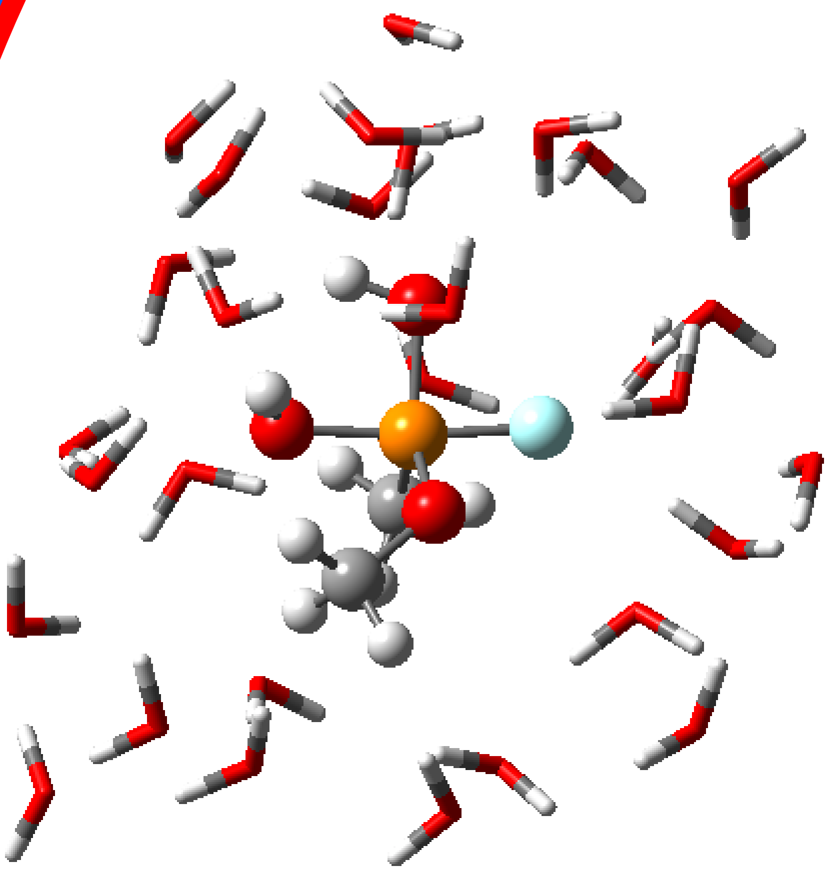
R and S propyl

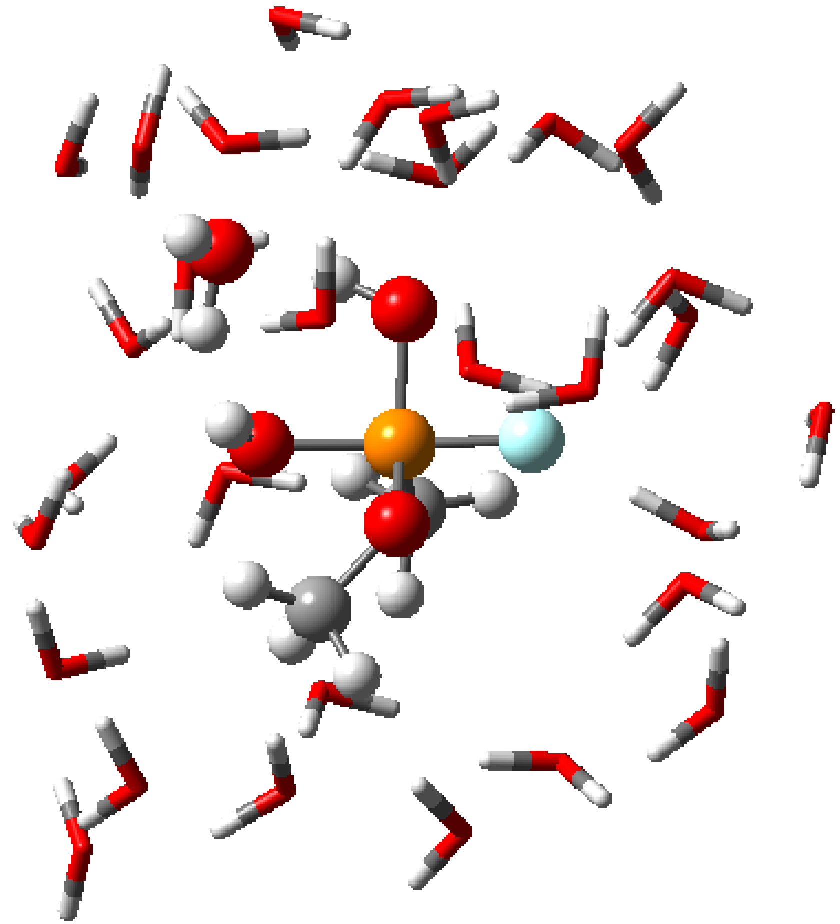
2-Water Hydrolysis of Methyl Methylphosphonofluoridate

B3LYP/6-311+G(2d,2p)

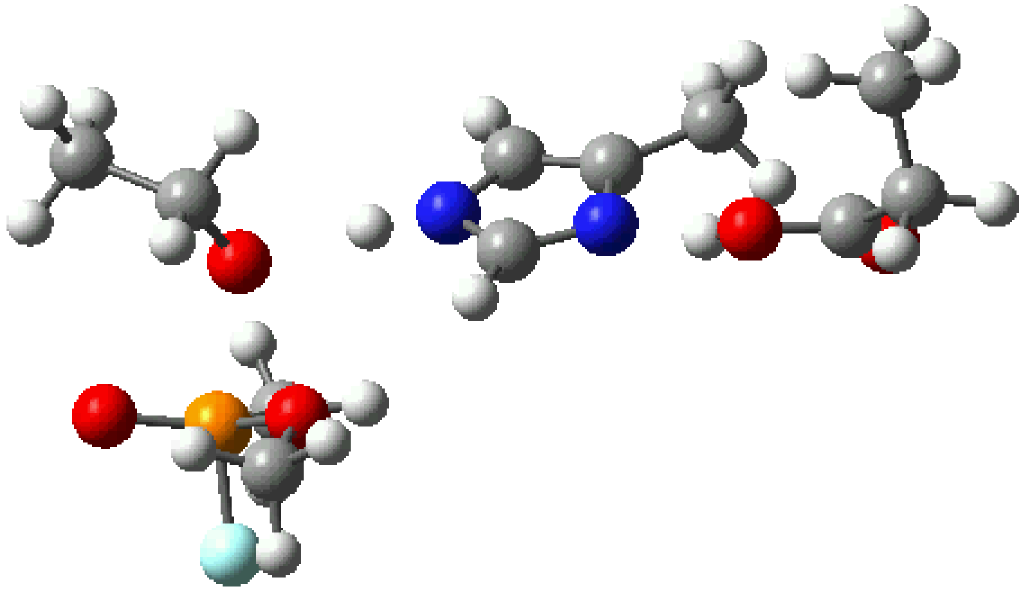


30-Water + 1&2 Water Hydrolysis of Methyl Methylphosphonofluoridate





G98
QM (TS)



HF/6-31G*

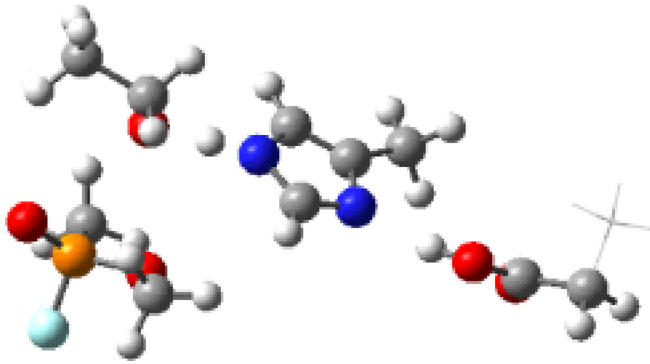
	TS
P-O_{SER}	2.49
O_{SER} - H_{SER}	1.56
H_{SER} - N_{HIS}	1.06
N_{HIS} - H_{HIS}	1.78
H_{HIS} -O_{GLU}	0.98
SSHB	2.76

Viragh C, Harris TK, Reddy PT, Massiah MA, Mildvan AS, Kovach IM (2000) Biochemistry 39:16200-16205

O_{GLU} – N_{HIS} SSHB = 2.64±0.04Å ([1]H NMR studies)

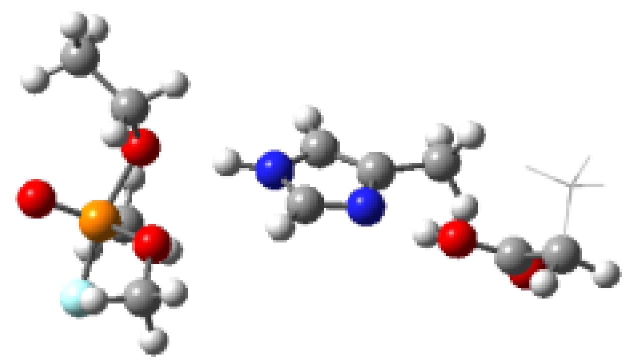
ONIOM/G98
QM/QM

oniom=(hf/6-31G*:hf/sto-3G)



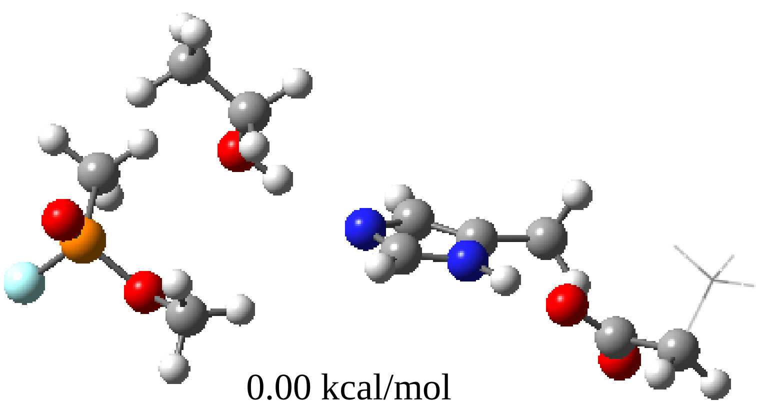
26.40 kcal/mol

SSHB = 2.74



16.35 kcal/mol

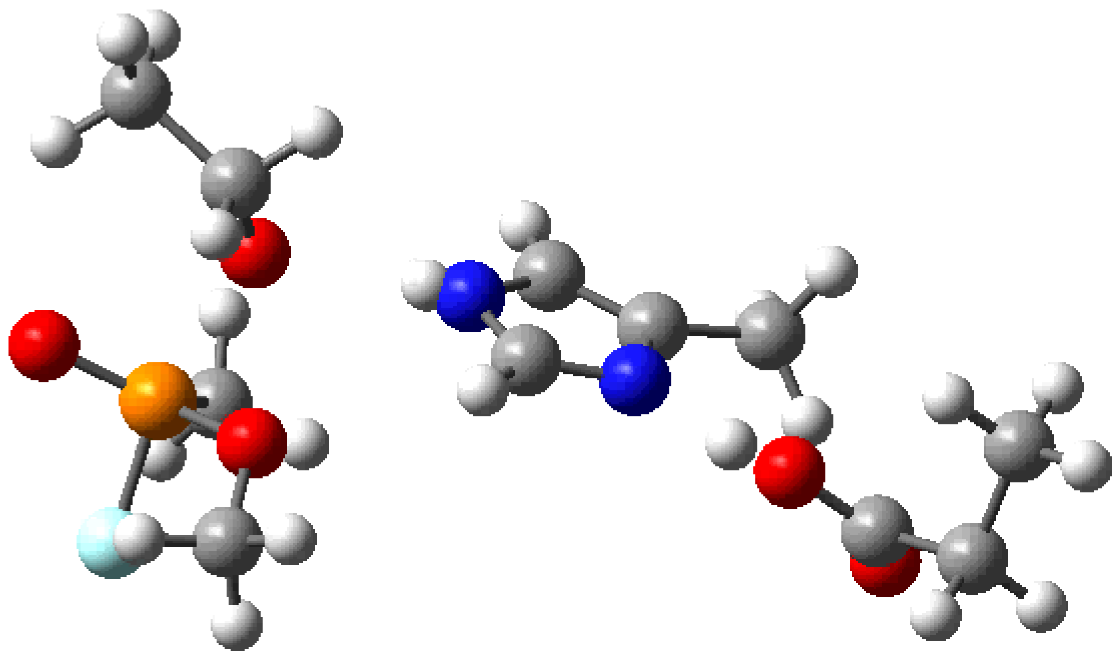
SSHB = 2.78



0.00 kcal/mol

SSHB = 2.73

G98
QM (TS)



B3LYP/6-31G(d,p)

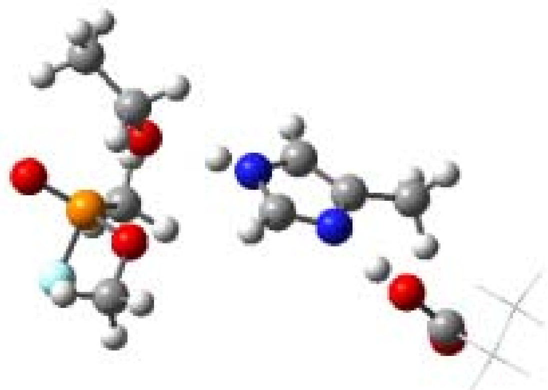
	TS
P-O_{SER}	2.11
O_{SER} - H_{SER}	1.22
H_{SER} - N_{HIS}	1.27
N_{HIS} - H_{HIS}	1.44
H_{HIS} -O_{GLU}	1.09
SSHB	2.53

Viragh C, Harris TK, Reddy PT, Massiah MA, Mildvan AS, Kovach IM (2000) Biochemistry 39:16200-16205

O_{GLU} – N_{HIS} SSHB = 2.64±0.04Å (^{1}H NMR studies)

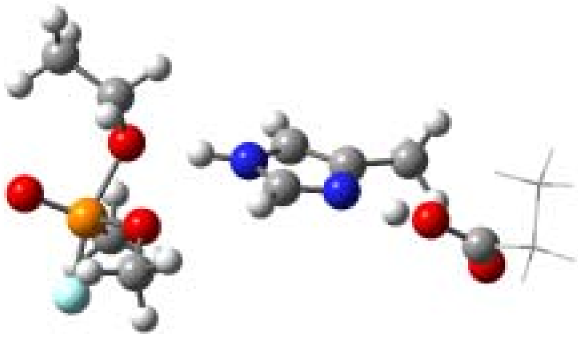
ONIOM/G98
QM/QM

oniom=(B3LYP/6-31G(d,p):B3LYP/3-21G)**



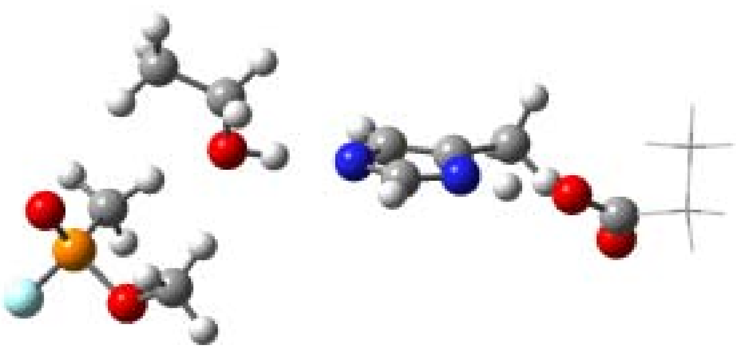
15.18 kcal/mol

SSHB = 2.52



12.62 kcal/mol

SSHB = 2.59

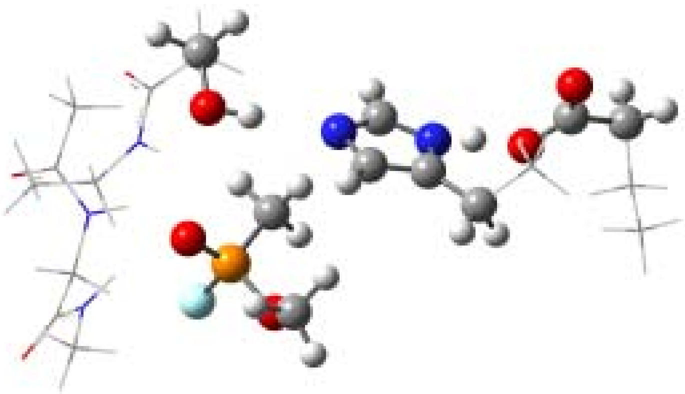


0.00 kcal/mol

SSHB = 2.59

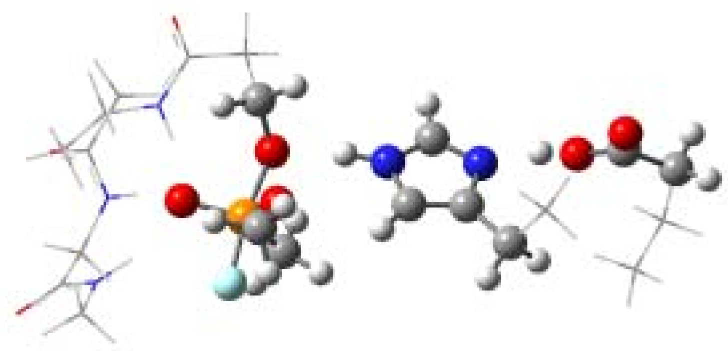
ONIOM/G98
QM/QM

oniom=(B3LYP/6-31G(d,p):B3LYP/3-21g)**



1.77 kcal/mol

SSHB = 2.59



0.00 kcal/mol

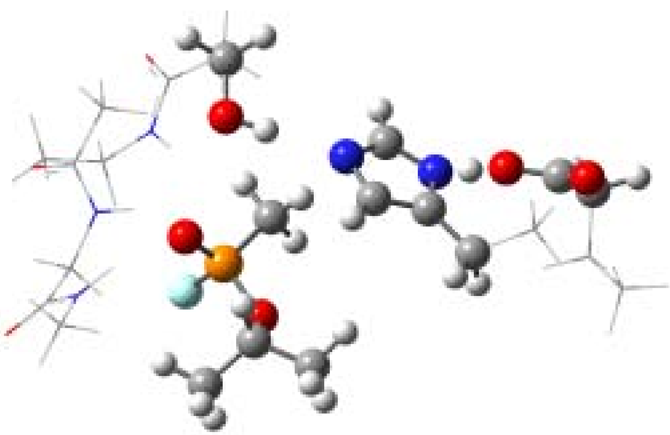
SSHB=2.61

Active site residues plus oxyanion hole

- **Agent O orients into oxyanion hole**
- **Charge transfer between agent and oxyanion hole**

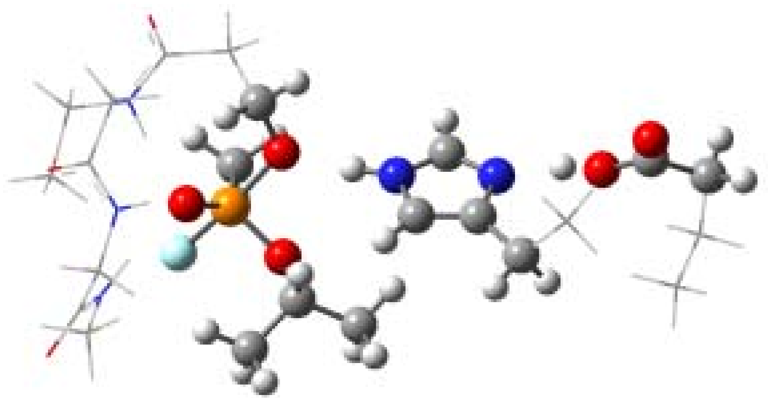
ONIOM/G98
QM/QM

oniom=(B3LYP/6-31G(d,p):B3LYP/3-21g)**



0.00 kcal/mol

SSHB = 2.58



11.03 kcal/mol

SSHB=2.63

Active site residues plus oxyanion hole

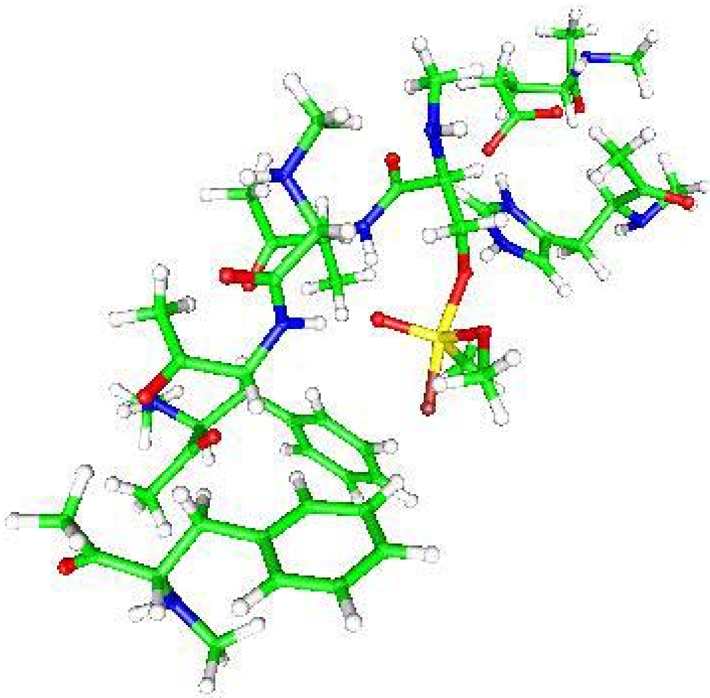
- **Sarin phosphonate group orients into oxyanion hole**
- **Charge transfer between agent and oxyanion hole**

Methyl-1-Methylphosphonofluoridate

Reaction	Level of Treatment	Transition State Barrier
1-Water hydrolysis 4-Membered TS	B3LYP / 6-311+G(2d,2p)	33.81 kcal/mol
2-Water hydrolysis 6-Membered TS	B3LYP / 6-311+G(2d,2p)	28.14 kcal/mol
Bare AChE catalytic triad	RHF / 6-31G*	26.43 kcal/mol
Bare AChE catalytic triad – ONIOM (Me – lower level)	RHF / 6-31G*: RHF/STO-3G	26.40 kcal/mol
Bare AChE catalytic triad	B3LYP / 6-31G*	15.27 kcal/mol
Bare AChE catalytic triad	B3LYP / 6-31G(d,p)	13.81 kcal/mol
Bare AChE catalytic triad	B3LYP / 6-311+G(2d,2p)	Have TS Work in Progress
Bare AChE catalytic triad – ONIOM (Ethyl – lower level)	B3LYP / 6-31G(d,p): PM3MM	15.66 kcal/mol
Bare AChE catalytic triad – ONIOM (Ethyl – lower level)	B3LYP / 6-31G(d,p): B3LYP/3-21G**	15.18 kcal/mol
Oxyanion hole + AChE catalytic triad	B3LYP/6-31G(d,p)	No TS Work in Progress
Oxyanion hole + AChE catalytic triad - ONIOM	B3LYP/6-31G(d,p): B3LYP/3-21G**	No TS Work in Progress

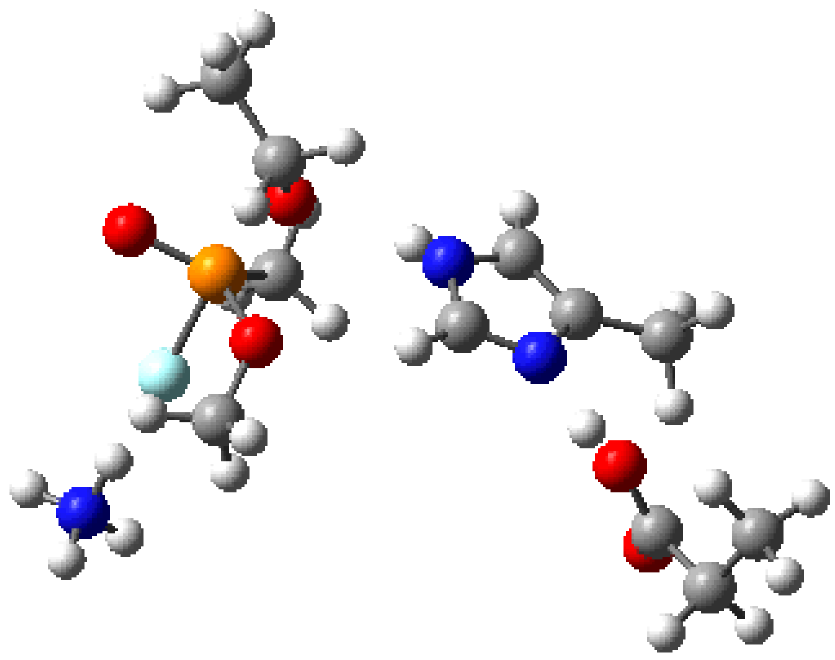
Methyl-1-Methylphosphonofluoridate

B3LYP/3-21G



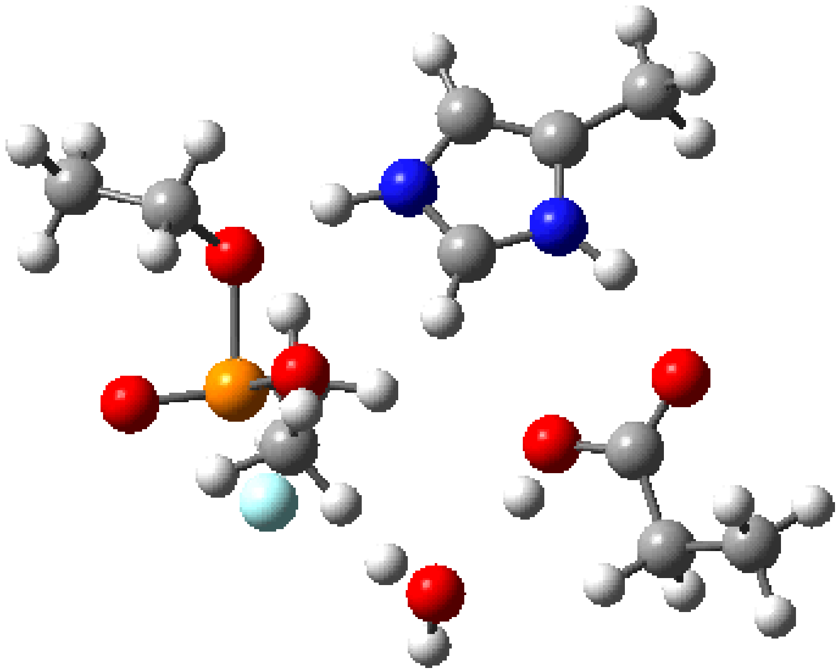
•LARGE calculation, 1 month on an SGI O2K
•F- not leaving?
•Maintain H-bonding with Oxyanion Hole
•Role of Acyl Pocket? Stereoselectivity? Aging?

QM/G98
F Leaving TS: w/ ammonium



TS shows simultaneous proton transfers, agent binding, F leaving

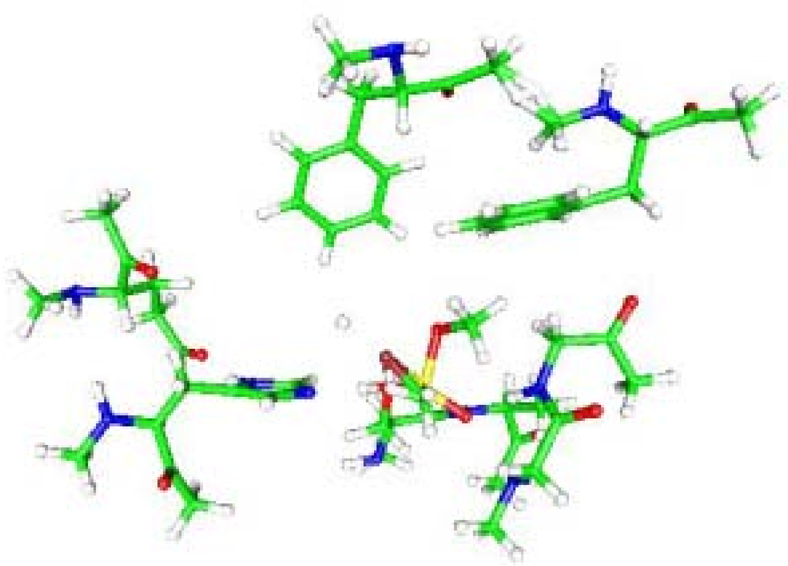
QM/G98
F Leaving TS: w/ hydronium



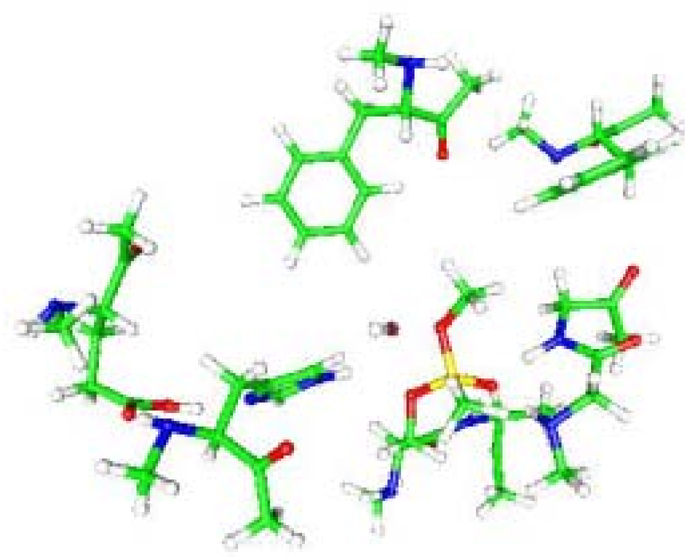
'The Anaconda' with multiple proton transfers, Agent binding, F leaving

QM/G98
F Leaving: Largest model w/ H

Oniom B3LYP/6-31g(d,p)/sto-3G

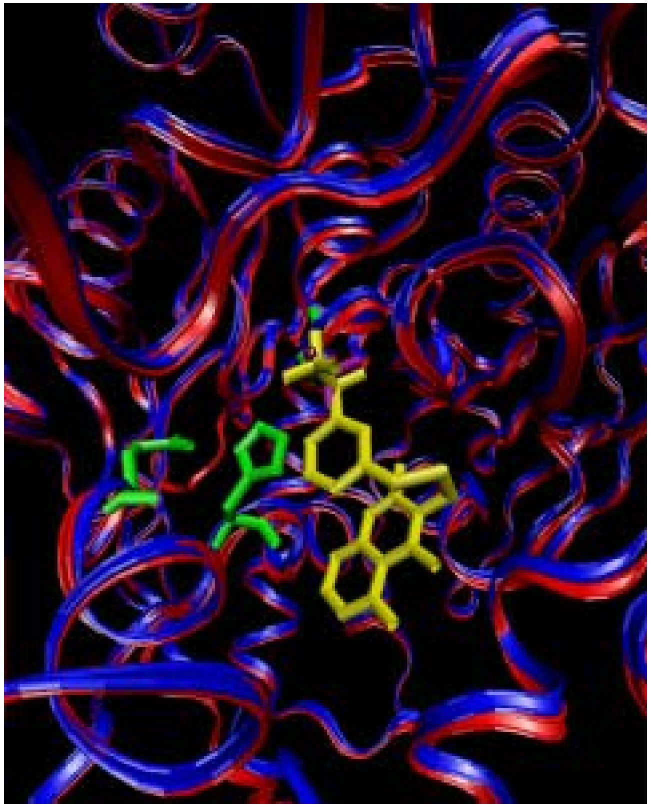


Initial Structure: Agent Unbound



Preliminary Final Structure: Agent Bound, HF leaving, tetragonal stucture

Conclusions/Future



Conclusions

- Preliminary analysis of sensitivity to details of model (FF, size, …)
- Validated model for bare enzyme
- SSHB and Reduction of barrier in enzyme
- Validated role of oxyanion hole
- Role of solvent, F leaving

Ongoing/Future:

- Aging and role of surrounding residues
- Stereoselectivity and role of Acyl Pocket
- Reversible binding
- Acetylcholine mechanism/Agent comparison

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